WEST Search History

DATE: Thursday, August 15, 2002

Set Name side by side	Query	Hit Count	Set Name result s t
	DB=USPT; PLUR=YES; OP=AND		
L1	5916764.pn.	1	L1
L2	dam	17863	L2
L3	L2 and methylase	199	L3
L4	$methylase	1291	L4
L5	L4 and l2	189	L5
L6	l3 or l5L5	202	L6
L7	l3 or l5	203	L7
L8	L7 and (gram or bacteri$ or microorganism or salmonella or bordetella or chlamydia or hemophilus or haemophilus or moraxella or mycobacter$)	197	L8
L9	l2 same l4	150	L9
L10	L9.clm.	16	L10
L11	bacteria or bacterium	78809	L11
L12	L11 near10 l2	21	L12
L13	$methylase	1291	L13
L14	L13 same (heterologous or foreign or second or additional or plurality or multiple or vaccine or immunogenic)	400	L14
L15	L13 same (heterologous or foreign or second or vaccine or immunogenic)	328	L15
L16	L13 same (heterologous or foreign)	109	L16

END OF SEARCH HISTORY

WEST Search History

DATE: Thursday, August 15, 2002

Set Name side by side	Query	Hit Count	Set Name result s t
	DB=USPT; PLUR=YES; OP=AND		
L1	dam.clm.	3037	L1
L2	L1 and salmonell$.clm.	2	L2
L3	salmonel$.ti,ab,clm.	804	L3
L4	L3 and dam	18	L4
L5	L4 not l2	16	L5
L6	dnamethyltransferase or dna-methyltransferase or dna-methyl-transferase or n-6-adenine-specific-dna-methylase or dna-methylase or (adenine near methylase) or deoxyadenosyl-methyltransferase or deoxy-adensyl-methyl-transferase or methyltransferase	836	L6
L7	L6.clm. and (salmonel$ or coli).clm.	12	L7

END OF SEARCH HISTORY

WEST Search History

DATE: Thursday, August 15, 2002

Set Name side by side	Query	Hit Count	Set Name result s t
	DB=USPT; PLUR=YES; OP=AND		
L1	5916764.pn.	1	L1
L2	dam	17863	L2
L3	L2 and methylase	199	L3
L4	$methylase	1291	L4
L5	L4 and l2	189	L5
L6	l3 or l5L5	202	L6
L7	l3 or l5	203	L7
L8	L7 and (gram or bacteri$ or microorganism or salmonella or bordetella or chlamydia or hemophilus or haemophilus or moraxella or mycobacter$)	197	L8
L9	l2 same l4	150	L9
L10	L9.clm.	16	L10
L11	bacteria or bacterium	78809	L11
L12	L11 near10 l2	21	L12

END OF SEARCH HISTORY

Heidelberg J.F., Eisen J.A., Nelson W.C., Clayton R.A., Gwinn M.L., Dodson R.J., Haft D.H., Hickey E.K., Peterson J.D., Umayam L.A., Gill S.R., Nelson K.E., Read T.D., Tettelin H., Richardson D., Ermolaeva M.D., Vamathevan J., Bass S., Qin H., Dragoi I., Sellers P., McDonald L., Utterback T., Fleischmann R.D., Nierman W.C., White O., Salzberg S.L., Smith H.O., Colwell R.R., Mekalanos J.J., Venter J.C., Fraser C.M.;
"DNA sequence of both chromosomes of the cholera pathogen Vibrio cholerae.";
Nature 406:477-483(2000).

Comments

- ***FUNCTION***: THIS ENZYME METHYLATES DNA WITHIN THE SEQUENCE GATC. DIRECTLY INVOLVED IN METHYL-DIRECTED DNA MISMATCH REPAIR *(BY SIMILARITY)*.
- ***CATALYTIC ACTIVITY***: S-adenosyl-L-methionine + DNA adenine = S-adenosyl-L-homocysteine + DNA 6-methylaminopurine.
- ***CAUTION***: REF.1 SEQUENCE DIFFERS FROM THAT SHOWN DUE TO NUMEROUS FRAMESHIFT ERRORS.

Copyright

Cross-references

EMBL	X67820; CAA48031.1; ALT_FRAME.	[EMBL / GenBank / DDBJ] [CoDingSequence]
	AF274317; AAG23174.1; -.	[EMBL / GenBank / DDBJ] [CoDingSequence]
	AE004329; AAF95767.1; -.	[EMBL / GenBank / DDBJ] [CoDingSequence]
HSSP	P04043; 2DPM. [HSSP ENTRY / PDB]	
REBASE	4762; M.VchADamP.	
TIGR	VC2626; -.	
InterPro	IPR002294; D12N6_mtfrase.	
	IPR002052; N6_Mtase.	
	Graphical view of domain structure.	
Pfam	PF02086; MethyltransfD12; 1.	
PRINTS	PR00505; D12N6MTFRASE.	
TIGRFAMs	TIGR00571; dam; 1.	
PROSITE	PS00092; N6_MTASE; 1.	
ProDom	[Domain structure / List of seq. sharing at least 1 domain].	
BLOCKS	Q08318.	
ProtoNet	Q08318.	
ProtoMap	Q08318.	
PRESAGE	Q08318.	
DIP	Q08318.	
ModBase	Q08318.	
SWISS-2DPAGE	GET REGION ON 2D PAGE.	

Keywords

Transferase; **Methyltransferase**; **DNA replication**; **Complete proteome**.

Features

None

Sequence information

Length: **277** **AA** Molecular weight: **31623** **Da** CRC64: **48D831240F9E7C60** [This is a checksum on the sequence]

```
        10         20         30         40         50         60
         |          |          |          |          |          |
MKKQRAFLKW AGGKYSLVED IQRHLPEARE LVEPFVGAGS VFLNTDFERY LLADINPDLI

        70         80         90        100        110        120
         |          |          |          |          |          |
NFYNLLKTEP QAYIHEAKRW FVPENNRKEV YLDIRKQFNQ SDDAMFRSLA FLYMNRFGFN

       130        140        150        160        170        180
         |          |          |          |          |          |
GLCRYNKKGG FNVPFGSYKK PYFPEQELEF FAEKAQRATF ICASYGETFA RAQSDSVIYC

       190        200        210        220        230        240
         |          |          |          |          |          |
DPPYAPLSTT ANFTSYAGNG FTLDDQAALA DIAEKTAKER GISVLISNHD TTHTRRLYRG

       250        260        270
         |          |          |
AQLNVVKANR TISRNGAGRN KVDELLALFT PHLSSQA
```

Q08318 in FASTA format

View entry in original SWISS-PROT format
View entry in raw text format (no links)
Report form for errors/updates in this SWISS-PROT entry

Direct BLAST submission at EMBnet-CH/SIB (Switzerland)

Direct BLAST submission at NCBI (Bethesda, USA)

ScanProsite, MotifScan

Sequence analysis tools: ProtParam, ProtScale, Compute pI/Mw, PeptideMass, PeptideCutter, Dotlet (Java)

Feature table viewer (Java)

Search the SWISS-MODEL Repository

☐ 1: Mol Genet Metab 1998 Nov;65(3):191-6 Related Articles, Books, LinkOut

ELSEVIER SCIENCE FULL-TEXT ARTICLE

Regulation of uropathogenic Escherichia coli adhesin expression by DNA methylation.

Hale WB, van der Woude MW, Braaten BA, Low DA.

Department of Pathology, University of Utah Health Sciences Center, Salt Lake City, Utah, 84132, USA.

Pap pili play an important role in the pathogenesis of upper urinary tract infections by enabling uropathogenic Escherichia coli to adhere to host epithelial cells. Pap pili are coded for by the pyelonephritis-associated pili (pap) operon, which consists of 11 genes required for the expression and assembly of Pap pili. Expression of Pap pili is regulated by a phase variation mechanism in which the pili expression state of the bacterial population is skewed between phase-on (expression positive) and phase-off (expression negative) states. Pap phase variation is controlled by the cooperative binding of leucine-responsive regulatory protein (Lrp) to two sets of Lrp binding sites in the upstream regulatory region of the pap operon. A single GATC sequence, which is the target site for deoxyadenosine methylase (Dam), is centrally located within each Lrp binding region. Dam plays a critical role in the expression of Pap pili via the formation of DNA methylation patterns. Methylation of GATC-I reduced the affinity of Lrp for pap DNA by about twofold. Conversely, Lrp specifically blocked methylation of pap GATC-I in vitro. These data support the hypothesis that Lrp and Dam compete for binding to GATC-I, and are consistent with previous results indicating that methylation of GATC-I is important for stability of the phase-off state.

PMID: 9851883 [PubMed - indexed for MEDLINE]

i686-pc-linux-gnu Jul 16 2002 16:34:53

NiceZyme View of ENZYME: EC 2.1.1.72

Official Name

Site-specific DNA-methyltransferase (adenine-specific).

Alternative Name(s)

N-6 adenine-specific DNA methylase.
Modification methylase.
Restriction-modification system.

Reaction catalysed

```
    S-adenosyl-L-methionine
 +  DNA adenine
<=>
    S-adenosyl-L-homocysteine
 +  DNA 6-methylaminopurine
```

Comments

- This is a large group of enzymes, the majority of which, with enzymes of similar site specificity listed as EC 3.1.21.3, EC 3.1.21.4 and EC 3.1.21.5, form so-called restriction- modification systems.
- See the REBASE database for a complete list of these enzymes: http://www.neb.com/rebase/

Cross-References

PROSITE	PDOC00087
BRENDA	2.1.1.72
EMP/PUMA	2.1.1.72
WIT	2.1.1.72
KYOTO UNIVERSITY LIGAND CHEMICAL DATABASE	2.1.1.72
IUBMB Enzyme Nomenclature	2.1.1.72
MEDLINE	Find literature relating to 2.1.1.72
	P21311, DMA7_ECOLI; P12427, DMA_BPT2 ; P04392, DMA_BPT4 ; P00475, DMA_ECOLI ; P44431, DMA_HAEIN ; P55893, DMA_SALTY ; P45454, DMA_SERMA ; O33844, DMA_TREPA ; Q08318, DMA_VIBCH ; P50179, ML21_LACLC; P50178, ML22_LACLC; P34720, MT1A_MORBO; P34721, MT1B_MORBO; P04043, MT21_STRPN; P09358, MT22_STRPN; P25240, MT57_ECOLI; P25201, MTA1_ACICA; P58284, MTA1_AZOBR; O30570, MTB1_BRUAB; P22772, MTB3_BACAR; P33563, MTBB_BACSU; P43423, MTC1_BACST; Q45971, MTC1_CAUCR; Q01511, MTC1_CHVN1;

☐ 1: Arch Microbiol 1998 Jun;169(6):530-3 Related Articles, Protein, Books, LinkOut

Microbiology

A temperature-sensitive DNA adenine methyltransferase mutant of Salmonella typhimurium.

Brawer R, Batista FD, Burrone OR, Sordelli DO, Cerquetti MC.

Centro de Estudios Farmacoligicos y Botanicos (CEFYBO-CONICET) and University of Buenos Aires, School of Medicine, Serrano 669, 1414 Buenos Aires, Argentina.

A temperature-sensitive mutant of Salmonella typhimurium was isolated earlier after transposon mutagenesis with Tn10d Tet. The mutant D220 grows well at 28 degreesC but has a lower growth rate and forms filaments at 37 degreesC. Transposon-flanking fragments of mutant D220 DNA were cloned and sequenced. The transposon was inserted in the dam gene between positions 803 and 804 (assigned allele number: dam-231 : : Tn10d Tet) and resulted in a predicted ten-amino-acid-shorter Dam protein. The insertion created a stop codon that led to a truncated Dam protein with a temperature-sensitive phenotype. The insertion dam-231 : : Tn10d Tet resulted in a dam "leaky" phenotype since methylated and unmethylated adenines in GATC sequences were present. In addition, the dam-231 : : Tn10d Tet insertion rendered dam mutants temperature-sensitive for growth depending upon the genetic background of the S. typhimurium strain. The wild-type dam gene of S. typhimurium exhibited 82% identity with the Escherichia coli dam gene.

PMID: 9575240 [PubMed - indexed for MEDLINE]

Display Abstract Sort Save Text Clip Add Order

☐ 1: Biol Chem 1998 Apr-May;379(4-5):475-80

Functional mapping of the EcoRV DNA methyltransferase by random mutagenesis and screening for catalytically inactive mutants.

Friedrich T, Roth M, Helm-Kruse S, Jeltsch A.

Institut fur Biochemie, Fachbereich Biologie, Justus-Liebig-Universitat, Giessen, Germany.

M.EcoRV is an alpha-adenine DNA methyltransferase. According to structure predictions, the enzyme consists of a catalytic domain, which has a structure similar to all other DNA-methyltransferases, and a smaller DNA-recognition domain. We have investigated this enzyme by random mutagenesis, using error-prone PCR, followed by selection for catalytically inactive mutants. 20 single mutants were identified that are completely inactive in vivo as His6- and GST-fusion proteins. 13 of them could be overexpressed and purified. All of these mutants are also inactive in vitro. 5 of the mutations are located near the putative binding site for a flipped adenine residue (C192R, D193G, E212G, W231R, N239H). All of these variants bind to DNA, demonstrating the importance of this region of the protein in catalysis. Only the W231R mutant could be purified with high yields. It binds to DNA and AdoMet and, thus, behaves like a bona fide active site mutant. According to the structure prediction Trp231 corresponds to Val121 in M.HhaI, which forms a hydrophobic contact to the flipped target cytosine. 4 of the remaining purified variants are located within a small region of the putative DNA-recognition domain (F115S, F117L, S121P, C122Y). F117L, S121P and C122Y are unable to bind to DNA, suggesting a critical role of this region in DNA binding. Taken together, these results are in good agreement with the structural model of M.EcoRV.

PMID: 9628340 [PubMed - indexed for MEDLINE]

☐ 1: Nucleic Acids Res 1997 Nov 1;25(21):4393-9 Related Articles, Books, LinkOut

FREE full text article at nar.oupjournals.org

Interaction of the phage T4 Dam DNA-[N6-adenine] methyltransferase with oligonucleotides containing native or modified (defective) recognition sites.

Malygin EG, Petrov NA, Gorbunov YA, Kossykh VG, Hattman S.

Institute of Molecular Biology, State Research Center of Virology, and Biotechnology 'Vector', Koltsovo, Novosibirsk Region 633159, Russia and Department of Biology, University of Rochester, Rochester, NY 14627-0211, USA.

The DNA-[N 6-adenine]-methyltransferase (Dam MTase) of phage T4 catalyzes methyl group transfer from S-adenosyl-l-methionine (AdoMet) to the N6-position of adenine in the palindromic sequence, GATC. We have used a gel shift assay to monitor complex formation between T4 Dam and various synthetic duplex oligonucleotides, either native or modified/defective. The results are summarized as follows. (i) T4 Dam bound with approximately 100-fold higher affinity to a 20mer specific (GATC-containing) duplex containing the canonical palindromic methylation sequence, GATC, than to a non-specific duplex containing another palindrome, GTAC. (ii) Compared with the unmethylated duplex, the hemimethylated 20mer specific duplex had a slightly increased (approximately 2-fold) ability to form complexes with T4 Dam. (iii) No stable complex was formed with a synthetic 12mer specific (GATC-containing) duplex, although T4 Dam can methylate it. This indicates that there is no relation between formation of a catalytically competent 12mer-Dam complex and one stable to gel electrophoresis. (iv) Formation of a stable complex did not require that both strands be contiguous or completely complementary. Absence of a single internucleotide phosphate strongly reduced complex formation only when missing between the T and C residues. This suggests that if T4 Dam makes critical contact(s) with a backbone phosphate(s), then the one between T and C is the only likely candidate. Having only one half of the recognition site intact on one strand was sufficient for stable complex formation provided that the 5'G.C base-pairs be present at both ends of the palindromic, GATC. Since absence of either a G or C abolished T4 Dam binding, we conclude that both strands are

☐ 1: Crit Rev Biochem Mol Biol 1996 Dec;31(5-6):361-80

Chemistry and biology of DNA methyltransferases.

Ahmad I, Rao DN.

Department of Biochemistry, Indian Institute of Science, Bangalore, India.

Recognition of a specific DNA sequence by a protein is probably the best example of macromolecular interactions leading to various events. It is a prerequisite to understanding the basis of protein-DNA interactions to a better insight into fundamental processes such as transcription, replication, repair, and recombination. DNA methyltransferases with varying sequence specificities provide an excellent model system for understanding the molecular mechanism of specific DNA recognition. Sequence comparison of cloned genes, along with mutational analyses and recent crystallographic studies, have clearly defined the functions of various conserved motifs. These enzymes access their target base in an elegant manner by flipping it out of the DNA double helix. The drastic protein-induced DNA distortion, first reported for HhaI DNA methyltransferase, appears to be a common mechanism employed by various proteins that need to act on bases. A remarkable feature of the catalytic mechanism of DNA (cytosine-5) methyltransferases is the ability of these enzymes to induce deamination of the target cytosine in the absence of S-adenosyl-L-methionine or its analogs. The enzyme-catalyzed deamination reaction is postulated to be the major cause of mutational hotspots at CpG islands responsible for various human genetic disorders. Methylation of adenine residues in Escherichia coli is known to regulate various processes such as transcription, replication, repair, recombination, transposition, and phage packaging.

Publication Types:
- Review
- Review, Academic

PMID: 8994802 [PubMed - indexed for MEDLINE]

NiceProt View of SWISS-PROT: Q08318

Printer-friendly view | Quick BlastP search

[General] [Name and origin] [References] [Comments] [Cross-references] [Keywords] [Features] [Sequence] [Tools]

General information about the entry

Entry name	**DMA_VIBCH**
Primary accession number	**Q08318**
Secondary accession number	Q9KNV4
Entered in SWISS-PROT in	Release 32, November 1995
Sequence was last modified in	Release 40, October 2001
Annotations were last modified in	Release 41, June 2002

Name and origin of the protein

Protein name	**DNA adenine methylase**
Synonyms	**EC 2.1.1.72** **Deoxyadenosyl-methyltransferase** **DNA adenine methyltransferase** **M.VchADam**
Gene name	**DAM** or **VC2626**
From	Vibrio cholerae [TaxID: 666]
Taxonomy	Bacteria; Proteobacteria; gamma subdivision; Vibrionaceae; Vibrio.

References

[1] SEQUENCE FROM NUCLEIC ACID.
MEDLINE=94171081; PubMed=8125341; [NCBI, ExPASy, EBI, Israel, Japan]
Bandyopadhyay R., Das J.;
"The DNA adenine methyltransferase-encoding gene (dam) of Vibrio cholerae.";
Gene 140:67-71(1994).

[2] SEQUENCE FROM NUCLEIC ACID.
STRAIN=Classical Ogawa 395 / ATCC 39541 / Serotype O1;
MEDLINE=21562622; PubMed=11705940; [NCBI, ExPASy, EBI, Israel, Japan]
Julio S.M., Heithoff D.M., Provenzano D., Klose K.E., Sinsheimer R.L., Low D.A., Mahan M.J.;
"DNA adenine methylase is essential for viability and plays a role in the pathogenesis of Yersinia pseudotuberculosis and Vibrio cholerae.";
Infect. Immun. 69:7610-7615(2001).

[3] SEQUENCE FROM NUCLEIC ACID.
STRAIN=El Tor N16961 / Serotype O1;
MEDLINE=20406833; PubMed=10952301; [NCBI, ExPASy, EBI, Israel, Japan]

Search in SWISS-PROT and TrEMBL for: dam methylase

SWISS-PROT Release 40.26 of 13-Aug-2002
TrEMBL Release 21.7 of 09-Aug-2002

- Number of sequences found in SWISS-PROT$_{(9)}$ and TrEMBL$_{(7)}$: **16**
- Note that the selected sequences can be saved to a file to be later retrieved; to do so, go to the bottom of this page.
- For more directed searches, you can use the Sequence Retrieval System SRS.

Search in SWISS-PROT: There are matches to 9 out of 112892 entries

DMA7_ECOLI (**P21311**)
Retron EC67 DNA adenine methylase (EC 2.1.1.72) (ORF1-EC67 DAM) (M.Eco67Dam). - Escherichia coli

DMA_BPT2 (**P12427**)
DNA adenine methylase (EC 2.1.1.72) (Deoxyadenosyl-methyltransferase) (M.EcoT2Dam). {GENE: DAM} - Bacteriophage T2

DMA_BPT4 (**P04392**)
DNA adenine methylase (EC 2.1.1.72) (Deoxyadenosyl-methyltransferase) (M.EcoT4Dam). {GENE: DAM} - Bacteriophage T4

DMA_ECOLI (**P00475**)
DNA adenine methylase (EC 2.1.1.72) (Deoxyadenosyl-methyltransferase) (DNA adenine methyltransferase) (M.EcoDam). {GENE: DAM OR B3387 OR Z4740 OR ECS4229} - Escherichia coli, Escherichia coli O157:H7

DMA_HAEIN (**P44431**)
DNA adenine methylase (EC 2.1.1.72) (Deoxyadenosyl-methyltransferase) (DNA adenine methyltransferase) (M.HindIV). {GENE: DAM OR HINDIVM OR HI0209} - Haemophilus influenzae

DMA_SALTY (**P55893**)
DNA adenine methylase (EC 2.1.1.72) (Deoxyadenosyl-methyltransferase) (DNA adenine methyltransferase) (M.StyDam). {GENE: DAM OR STM3484 OR STY4312} - Salmonella typhimurium, Salmonella typhi

DMA_SERMA (**P45454**)
DNA adenine methylase (EC 2.1.1.72) (Deoxyadenosyl-methyltransferase) (DNA adenine methyltransferase) (M.SmaII). {GENE: DAM} - Serratia marcescens

DMA_TREPA (**O33844**)
DNA adenine methylase (EC 2.1.1.72) (Deoxyadenosyl-methyltransferase) (DNA adenine methyltransferase) (M.TpaI). {GENE: DAM OR TP0810} - Treponema pallidum
DMA_VIBCH (**Q08318**)
DNA adenine methylase (EC 2.1.1.72) (Deoxyadenosyl-methyltransferase) (DNA adenine methyltransferase) (M.VchADam). {GENE: DAM OR VC2626} - Vibrio cholerae

---

Search in TrEMBL: There are matches to 7 out of 668930 entries

Q8SBE9
DNA adenine methylase {GENE:DAM} - Bacteriophage SfV (Shigella flexneri bacteriophage V)
Q8W672
DNA adenine methylase (Fragment) {GENE:DAM} - Bacteriophage RB49
Q97DW2
Site-specific DNA methylase dam {GENE:CAC3358} - Clostridium acetobutylicum
Q9CLK4
Dam (DNA adenine methylase) {GENE:DAM OR PM1222} - Pasteurella multocida
Q9F7U9
DNA adenine methylase (EC 2.1.1.72) {GENE:DAM OR YPO0154} - Yersinia pestis, Yersinia pseudotuberculosis
Q9L5T8
Putative DNA adenine methylase (dam) {GENE:R0041} - Salmonella typhi [Plasmid R27]
Q9X3Y7
Putative DNA adenine methylase {GENE:DAM} - Neisseria meningitidis

---

New Search
in SWISS-PROT/TrEMBL by AC, ID, description, gene name, organism
Please do NOT use any boolean operators (and, or, etc.)

If you would like to retrieve all the entries contained in this list , you can enter a file name. These entries will then be saved to a file under this name in the directory `outgoing` of the ExPASy anonymous ftp server, from where you can download it. (Please note that this temporary file will only be kept for 1 week.)

File name:

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WEST

Generate Collection Print

L5: Entry 2 of 16 File: USPT

DOCUMENT-IDENTIFIER: US 6277608 B1
TITLE: Recombinational cloning using nucleic acids having recombination sites

Detailed Description Text (194):
Restriction enzyme DpnI recognizes the sequence GATC and cuts that sequence only if the A is methylated by the dam methylase. Most commonly used E. coli strains are dam.sup.+. Expression of DpnI in dam.sup.+ strains of E. coli is lethal because the chromosome of the cell is chopped into many pieces. However, in dam.sup.- cells expression of DpnI is innocuous because the chromosome is immune to DpnI cutting.

CLAIMS:

11. The method of claim 10, wherein said prokaryotic vectors comprise vectors which replicate in bacteria of the genus Escherichia, Salmonella, Bacillus, Streptomyces or Pseudemonas.

WEST

☐ Generate Collection

L5: Entry 9 of 16 File: USPT

DOCUMENT-IDENTIFIER: US 5672345 A
TITLE: Selective maintenance of a recombinant gene in a population of vaccine cells

Detailed Description Paragraph Table (1):
TABLE 1

Bacterial strains Strain Parent strain/ number plasmid Relevant genotype Derivation

A.

Escherichia coli strains X289 K-12/F.sup.- prototroph glnV42 Curtiss collection X2108 K-12/F.sup.- leu-50 tsx-98 proB59 HNO.sub.2 -induced Asd.sup.- mutant of X2087 .DELTA.69[lacZOPI] .DELTA.40[gal-uvrB] rpsL206 .DELTA.asdA4 argH70 X2230 K-12/F.sup.- thr-1 leu-6 proA2 his-4 metB1 dam-3 derivative of X2234 (M. G. lacY1 galK2 ara-14 tsx-33 thi-1 Marinus) thyA12 deoB6 supE44 dam-3 (mtl-1) LE392 K-12/F.sup.- lacY1 glnV44 .lambda..sup.- galK2 galT22 P. Leder tyrT58 metB1 hsdR514 trpR55 X2637 K-12/F.sup.- tsx-63 purB41 glnV42 .lambda..sup.- pyrF30 Plcml(X2108 .fwdarw. X660 with sel'n for bis-53 tte-1 .DELTA.asdA4 xyl-14 AroB.sup.+ Asd.sup.- cycB2 cycA1 X2842 K-12/F.sup.- prototroph, suppressor-free Curtiss collection X2978 K-12/F.sup.- tax-63 purE41 glnv42 .lambda..sup.- pyrF30 P1L4(X2842::Tn10 library) .fwdarw. X2637 his-53 tte-1 zhf-2::Tn10 with sel'n for Tc.sup.r Asd.sup.+ xyl-14 cycB2 cycA1 X2979 K-12/F.sup.- tsx-63 purE41 glnv42 .lambda..sup.- pyrF30 P1L4(X2978 .fwdarw. X2637 with sel'n for his-53 tte-1 .DELTA.asdA4 zhf-2::Tn10 Tc.sup.r xyl-14 cycB2 cycA1 X2981 K-12/F.sup.- .DELTA.41[proB-lacYZ] glnV42 .lambda..sup.- tte-1 P1L4(X2979) .fwdarw. X354 with sel'n for .DELTA.asdA4 zhf-2::Tn10 cycA1 Tc.sup.r Asd.sup.- X2984 K-12/F.sup.- .DELTA.41[proB-lacYZ] .lambda..sup.- tte-1 FA.sup.r Tc.sup.s derivative of X2981 .DELTA.[zhf-2::Tn10] cycA1 CC118 K-12/F.sup.- araD139 .DELTA.(ara, leu)7697 .DELTA.lacX74 Manoil and Beckwith.sup.a phoA.DELTA.20 galE galR recA1 rpsE argE.sub.am rpoB thi X6094 K-12/F.sup.- lacY1 glnV44 .lambda..sup.- galK2 galV22 P1L4(X2981) .fwdarw. X2602 with sel'n for tyrT58 .DELTA.asdA4 zhf-2::Tn10 metB1 Tc.sup.r Asd.sup.- hsdR514 trpR55 JM83 K-12/F.sup.- ara .DELTA.[lac-pro] rpsL thi .phi.80dlacZ Viera and Messing.sup.b .DELTA.M15 X6096 K-12/F.sup.- ara .DELTA.[lac-pro] rpsL .DELTA.asdA4 P1L4(X2981) .fwdarw. JM83 zhf-2::Tn10 thi .phi.80dlacZ .DELTA.M15 with sel'n for Tc.sup.r Asd.sup.- X6097 K-12/F.sup.- ara .DELTA.[lac-pro] rpsL .DELTA.asdA4 Fa.sup.r Tc.sup.s derivative of X6096 .DELTA.[zhf-2::Tn10] thi .phi.80dlacZ .DELTA.M15 Y1090 K-12/F.sup.- .DELTA.araD139 .DELTA.lacU169 .DELTA.lon Promega Biotech tyrT trpC22::Tn10 rpsL hsdR (pBR322 lacI.sup.q) B. Salmonella typhimurium strains X3000 LT2-Z/pStLT100 prototroph, suppressor-free Curtiss collection X3008 LT2-Z/pStLT100 asdA15 HNO.sub.2 -induced Asd.sup.- mutant of X3000 X3013 LT2-Z/pStLT100 zhf-1::Tn10 from P22(X3000::Tn10 library) .fwdarw. X3008 with sel'n for Tc.sup.r Asd.sup.+ X3021 LT2-Z/pStLT100 .DELTA.[zhf-1::Tn10] .DELTA.asdA1 P22.sup.a FA.sup.r Tc.sup.s derivative of X3013 X3105 SR-11/pStSR100 zhf-1::Tn10 P22.sup.i P22(X3013) .fwdarw. X3041 with sel'n for Tc.sup.r X3115 SR-11/pStSR100 .DELTA.asdA3 P22.sup.i ATCC 39961 (FA.sup.r sel'n of X3105) SGSC452 LT2-Z/pStLT100 leu hsdLT galEtrpD2 rpsL120 Bullas and Ryu.sup.c metE551 metA22 hsdSA bsdSB ilv DB4673= LT2-Z/pStLT100 galE496 trpB2 flaA66 rpsL120 Palva and Liljestrom.sup.d TS736 xyl-404 val metE551 metA22 .DELTA.malB hsdSA29 hsdL6/F'112 (malE malF malK lamB from E. coli) X3385 LT2-Z hsdL6 galE596 trpB2 flaA66 cured derivative of AS68 (E. T. Palva) his-6165 rpsL120 xyl-404 metE551 metA22 lamB.sup.+ (E. coli) .DELTA.zja:Tn10 hsdSA29 val X3457 LT2-Z/pStLT100 nadA540::Tn10

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L7: Entry 11 of 12 File: USPT

DOCUMENT-IDENTIFIER: US 5491060 A
TITLE: Mutant strain of E. coli for detection of methyltransferase clones

CLAIMS:

1. A method for detecting E. coli bacterial cells containing a cloned methyltransferase gene, said cells expressing a methyltransferase enzyme, comprising:

transforming an E. coli bacterial cell having a gene encoding the production of a temperature sensitive DNA restriction enzyme reactive with DNA methylated by said methyltransferase, by combining said cell with a plasmid encoding said methyltransferase, said enzymae being active at a first temperature range termed the non-permissive temperature and inactive at a second temperature range termed the permissive temperature,

wherein cells which grow at said permissive temperature range and do not grow at said non-permissive temperature range are expressing said methyltransferase.

2. The method of claim 1, wherein the gene sequence for said methyltransferase is known.

3. The method of claim 1, wherein the gene sequence for said methyltransferase is unknown.

4. A method for producing methyltransferase, comprising:

transforming E. coli bacterial cells having a gene encoding the production of a temperature-sensitive DNA restriction enzyme reactive with DNA methylated by said methyltransferase, by combining said bacterial cells with a plasmid encoding said methyltransferase, said temperature sensitive restriction enzyme being inactive at a temperature range at which the cells can grow and multiply,

recovering the methyltransferase expressed by said cells.

6. The method of claim 4, wherein said bacterial cells are grown on a nutrient medium, and said methyltransferase is recovered from said medium.

7. The method of claim 4, wherein said bacterial cells are harvested, and said methyltransferase is obtained directly from said cells.

8. A method of producing mutant, temperature sensitive E. coli bacteria capable of expressing a methyltransferase enzyme, comprising:

exposing E. coli bacteria having a restriction enzyme gene, selected from the group consisting of mcrA, mcrB and mrr, to a mutagen,

cultivating the bacteria surviving exposure to said mutagen at a first temperature range, termed the permissive temperature range, at which the gene corresponding to said restriction enzyme gene is inactive, but the bacteria continue to grow, and transforming said cultivated bacteria with a plasmid encoding a methyltransferase capable of methylating DNA to which said restriction enzyme is reactive,

wherein said cells which grow at said permissive temperature range, but not at said non-permissive temperature range express said methyltransferase.

13. An E. coli novel bacterial cell, having a genome comprising a plasmid which encodes a methyltransferase, said genome further comprising a gene encoding a temperature sensitive restriction enzyme reactive with methylated DNA produced by said methyltransferase, the restriction enzyme being active at a first, non-permissive temperature range, and inactive at a second, permissive temperature range, wherein-said bacterial cell can grow at said permissive temperature range, but not at said non-permissive temperature range.

14. The bacterial cell of claim 13, wherein said bacterial cell is an E. coli bacterial cell mutated by exposure to a mutagen.

15. The method of claim 1, wherein identification of a methyltransferase encoding transformant indicates the transformant also contains a gene encoding a restriction enzyme associated with said gene encoding said methyltransferase.

☐ 1: Genetics 1996 Sep;144(1):15-26

DNA adenine methylase mutants of Salmonella typhimurium and a novel dam-regulated locus.

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Mutants of Salmonella typhimurium lacking DNA adenine methylase were isolated; they include insertion and deletion alleles. The dam locus maps at 75 min between cysG and aroB, similar to the Escherichia coli dam gene. Dam- mutants of S. typhimurium resemble those of E coli in the following phenotypes: (1) increased spontaneous mutations, (2) moderate SOS induction, (3) enhancement of duplication segregation, (4) inviability of recA and dam recB mutants, and (5) suppression of the inviability of the dam recA and dam recB combinations by mutations that eliminate mismatch repair. However, differences between S. typhimurium and E. coli dam mutants are also found: (1) S. typhimurium dam mutants do not show increased UV sensitivity, suggesting that methyl-directed mismatch repair does not participate in the repair of UV-induced DNA damage in Salmonella. (2) S. typhimurium dam recJ mutants are viable, suggesting that the Salmonella RecJ function does not participate in the repair of DNA strand breaks formed in the absence of Dam methylation. We also describe a genetic screen for detecting novel genes regulated by Dam methylation and a locus repressed by Dam methylation in the S. typhimurium virulence (or "cryptic") plasmid.

PMID: 8878670 [PubMed - indexed for MEDLINE]

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